CONDUCTIVITY OF BACTERIAL CELLS

R. G. GREEN AND W. P. LARSON

From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis

During late years much has been written concerning electrical conductivity of cells as a method of measuring permeability of cell membranes. While there seems to be doubt as to the validity of this method in the minds of some, most writers on this subject accept the method as measuring permeability and draw conclusions from their results on this basis. Our experiments with conductivity of bacterial cells were begun with the idea of determining whether immunologic reactions could be demonstrated to bring about changes in the permeability of bacterial membranes. The progression of our experimental work on this problem led us to doubt more and more that we were measuring an actual change in permeability of the bacterial membranes to the ions carrying the electric current, and we seem to have found an explanatory analysis of our results much more complex than has been indicated by the conclusions of other investigators.

The fundamental principle that a solution containing cells has a higher resistance than the same solution without cells seems to have been established before 1900, being used by Fischer,[1] Roth,[2] Burgarsky and Tangl,[3] Stewart,[4], and Oker-Blom,[5] working with red blood cells in serums and salt solutions. Stewart [6] demonstrated that the resistance of a suspension of erythrocytes decreased on hemolysis, and McClendon [7] showed a decrease in the resistance of a suspension of echinoderm eggs on fertilization. This and similar experimental work by others has formed the basis for the more elaborate work which has been done in applying the conductivity method to the measurement of functional changes in permeability of cell membranes. The decrease observed in the resistance of a suspension of live cells on injury or death of the cells seems to be accepted by most investigators as a

Received for publication, Jan. 25, 1922.

* Aided by a grant from the National Dental Research Association.

1 Jahrb. d. Botanik, 1895, 27, p. 1.
2 Elect. Leitfähigkeit thierischer Flüssigkeiten, 1897.
3 Ztschr. f. Phys., 1897, 2, p. 297.
4 Jour. Physiol., 1899, 24, p. 356.
5 Pflüger's Arch., 1900, 79, p. 111 and 510.
6 Jour. Pharmacol. & Exper. Therap., 1910, 1, p. 49.
7 Am. Jour. Physiol., 1910, 27, p. 270.

quantitative measure of an increased conductance by the cells due to a change in the cell membrane. Osterhout [8] definitely subscribes to this view in his work with Laminaria and finds that the resistance of a cylinder of Laminaria disks in sea water drops to the resistance of an equal cylinder of sea water when the cells die. Gray [9] working with echinoderm eggs reports similar findings. Shearer [10] finds that when a suspension of B. coli in Ringer's solution is heated, the resistance drops to that of Ringer's solution alone. He states that the resistance is produced by the living state and that dead cells offer no more resistance than so much agar or gelatin. The idea that the observed changes in resistance are due to an increased permeability of the membrane to the ions carrying the electric current was not held by Stiles and Jorgensen,[11] and they pointed out that the exchange of salts between the cell interior and the liquid surrounding the cell must be taken into consideration. Stiles and Kidd [12] measured the osmosis of salts by measuring the conductivity of the solution surrounding plant tissues and considered this a measurement of permeability. However, experimental work taking into account all factors involved in this complex problem with consideration of the relative effects of each, seems at present to be lacking.

In our work, suspensions of bacteria were used. Results from the use of free cells suspended in a simple solution would seem to be more easily analyzed than those obtained from the use of mass tissue in that there is only one type of cell present and because there is no intercellular substance to complicate the interpretation of measurements. Bacterial cells may be preferred to eggs for this type of experimentation in that we are not sure the membrane of a dormant egg is comparable in properties of permeability to the membrane of a complete individual making use of its membrane by virtue of an active metabolism. Above all, with the use of suspended cells, the liquid may be separated from the cells and studied separately.

Our experiments were made with B. coli and Staphylococcus albus which were grown in Kolle flasks, washed off, centrifuged from the wash water and resuspended in salt solutions, the strength and character of which varied with other experimental conditions. The measurements were made with a modified Wheatstone bridge using an

[8] Science, 1912, 35, p. 112.
[9] Proc. Roy. Soc., B, 1916, 207, p. 481.
[10] Jour. Hygiene, 1919, 18, p. 360.
[11] Ann. Bot., 1915, 29, p. 611.
[12] Proc. Roy. Soc., B, 1919, 90, p. 448.

oscillating current of 4,000 cycles obtained from a Vreeland oscillator. Duplicate readings with radio-frequency from an audion bulb showed that variations in frequency do not produce variations in measured resistance and so introduce no error. Very delicate and accurate readings were obtainable by the use of a variometer in series with the electrolytic cell. The measurements were made in a water bath constant to $\frac{1}{25}$ degree Centigrade. The electrolytic cell was an L or boot shaped cell, the horizontal portion, which carried the electrodes, having a capacity of 1.5 mils.

The experiments here reported concern changes in resistance of suspensions of bacteria on death of the bacterial cells as produced by heat and liquor formaldehydi. Experiments were carried out in general according to the following technic:

The suspension of bacteria having been made in a salt solution of the desired strength, its resistance was measured by transferring 2 mils to the electrolytic cell. On replacing this, the suspension was centrifuged and the clear supernatant liquid obtained, the resistance of which was measured. A suspension was formed once more by means of a rotating glass stirrer until constant readings were obtained and the suspension resistance again measured as a check. The suspension was then heated at a definite temperature for a definite time which varied in different experiments. After a thorough mixing the resistance of the heated suspension was determined. Again centrifuging to obtain the supernatant liquid from the killed bacteria, its resistance was measured. After mixing the dead cells with the solution once more, the resistance of the suspension of dead bacteria was again obtained. The measurements of the unheated or live organisms before and after centrifuging showed that the operation did not bring about any change in resistance, so experimental error from that source seems to be eliminated.

For convenience the suspensions are designated as live or dead, the degree of heat applied being above the thermal death point for the bacteria used. The liquid suspending the cells is referred to as the menstruum live, or menstruum dead, depending on whether it was suspending live or dead cells when separated for measurement. The results of several experiments using B. coli are given. They are put in graphic form to show the relation of the different values at a glance. The ordinates represent resistance in ohms, the abscissas merely indicate the operation which brought about a change in the ordinates.

Fig. 1 represents the results obtained with the heating of B. coli in approximately 0.5% NaCl solution at 60 C. for 90 minutes.

Fig. 2 shows the changes in resistance which occurred when B. coli was heated in approximately 0.3% NaCl at 92 C. for 6 minutes. The

menstruum in these experiments was mainly an NaCl solution as specified, but the solution contained some calcium and other ions carried over from the nutrient medium.

From these data the following points are to be noted for subsequent discussion.

1. Coincident with a drop in resistance of the bacterial suspension, there is a marked drop in the resistance of the menstruum.

2. The dead bacterial cells act to raise the resistance of the solution in which they are suspended similar to live cells, but it would appear, on superficial consideration at least, to a lesser extent.

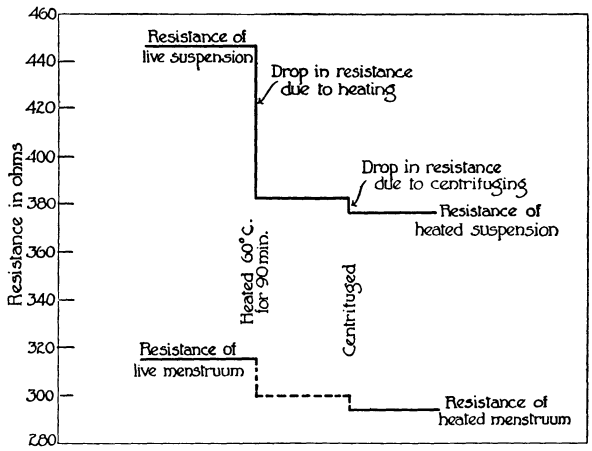

Fig. 1.—B. coli suspended in 0.5% NaCl solution and heated to 60 C. for 9 minutes.

3. After the cells are heated and the resistance of the suspension determined, a further drop in resistance is found to occur on centrifuging and remixing.

The first of these points has its explanation in the exosmosis of salts from the cells into the surrounding medium. This decrease in resistance of the menstruum is always included in the measurement of the cells plus the menstruum, and of course becomes an error when the drop in suspension resistance is interpreted as measuring increased penetration of the plasmic membrane to mobile ions. To eliminate this error it is evident that the concentration of salts inside and outside the cell must be balanced in such a manner that when the membrane of the cell is made completely permeable there will be no increase in the number of current carriers in the liquid between the cells. These

experimental conditions are probably never fulfilled. In our experiments we have found that bacteria growing on ordinary media store up salts to a greater concentration than is found in the media on which they are grown. On killing Spirogyra in the pond water in which they were rapidly growing, we likewise found a great exosmosis of the salts from the cells into their natural habitat. We have termed these diffusible salts the physiologic, in contradistinction to the structural salts which are bound up in body structure and demonstrated only by ash analysis. It is our ignorance of the conditions controlling or determining the concentration of these physiologic salts that makes this factor so difficult to estimate or eliminate in conductivity measurements.

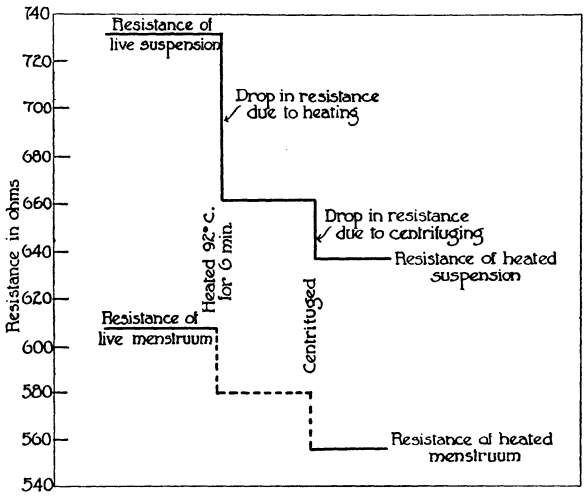

Fig. 2.—B. coli suspended in 0.3% NaCl solution and heated at 92 C. for 6 minutes.

The second point noted, that dead cells increase the resistance of a solution in which they are placed, is a constant finding in all of our experiments. A comparison with live cells made on the basis of solutions of equal resistance shows that the dead cells raise the resistance of the solution 75% or more of the amount observed when the same cells were alive. While this finding is contrary to the results of other investigators, we call attention to the simplicity of the technic used in our experiments. The suspension of bacteria was broken up by centrifuging and remixing repeatedly, the resistance of the solution alone and then the solution suspending the cells being measured each time. The

result was not affected by the degree of heat used, and findings were similar when the cells were killed by liquor formaldehydi.

We also noted that the heated suspension was different from the unheated in that a drop in resistance took place on centrifuging and remixing. A further drop did not take place on repeating the manipulation. It does not seem possible to say definitely whether this is due to a change in the menstruum or in the bacteria themselves. There are several possibilities. A change in permeability of the membrane due to centrifuging seems unlikely. It might be due to a decrease in the size of the bacteria, a possibility given in detail below. A

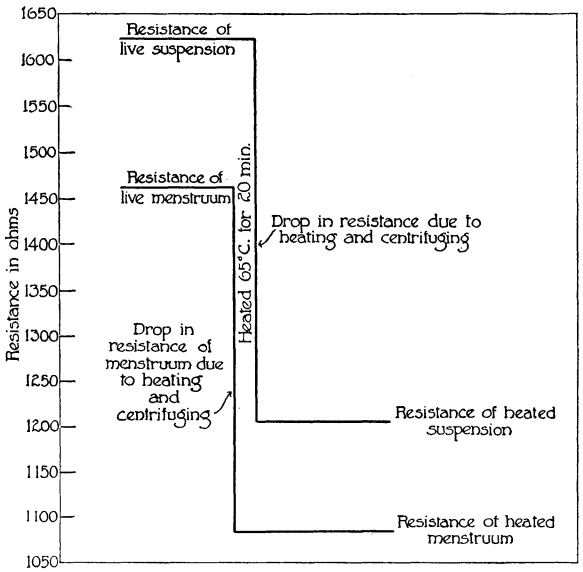

Fig. 3.—B. coli suspended in 0.7% NaCl solution and heated at 65 C. for 20 minutes.

third possibility is that there is a further exosmosis of salts from the bacteria into the surrounding menstruum due to the mechanical action of centrifuging. It is seen from the graphic representation of our results that we subscribe to the latter view as the explanation.

The quantitative effect of exosmosis must depend on the relation between the concentration of electrolytes within and around the cells. Figures 3, 4 and 5 are graphic representations of an experiment in which the same number of bacteria, grown under identical conditions, were killed by heat when suspended in equal quantities of NaCl solution varying in NaCl content.

A suspension of B. coli was divided into equal parts by weight and after centrifuging, NaCl solutions were substituted for the original menstruum in weighed amounts. This experiment again demonstrates the results previously discussed and illustrates the relation of the electrolyte concentration of the menstruum surrounding the cells to the results obtained by conductivity measurements. Fig. 4 is an example of experimental conditions being right to make the resistance of the menstruum plus the dead bacteria equal to the live menstruum alone, from which it might be erroneously assumed that dead cells offer little or no resistance if the heated menstruum were not separated and measured.

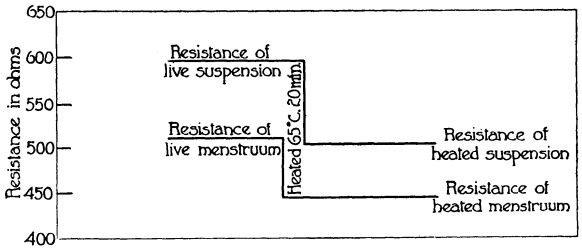

Fig. 4.—B. coli suspended in 0.3% NaCl solution and heated at 65 C. for 20 minutes.

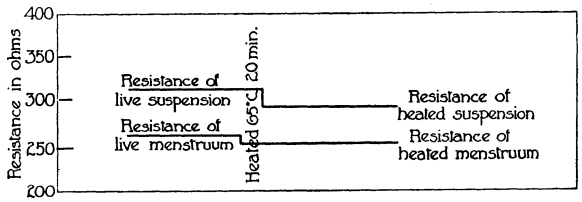

Fig. 5.—B. coli suspended in 0.1% NaCl solution and heated at 65 C. for 20 minutes.

Another important factor that must be considered in the interpretation of conductivity measurements is the variation in volume of cells if there is any. If it is true that cells store up salts to a greater concentration than is found in their liquid habitat, the resulting turgor must keep the cell expanded to a maximum volume. It might be expected that an exosmosis of salts might allow a shrinking of the volume of the cell and that this might be increased by the packing of the cells in centrifuging. Some support for this view is gained

from the common observation that the centrifuged volume of bacterial cells is much decreased after the cells are heated.

Direct measurements made on live and dead bacterial cells when prepared by both positive and negative stains show considerable decrease in the size of individual cells on death. The effect of a decrease in the size of the bacterial cells on death is that less space will be taken up between the electrodes by the dead bacterial bodies which we have found to have a higher resistance than the suspending solution, and there will be a corresponding decrease in total resistance due to this factor.

It appears that if the resistance of a suspension of bacteria decreases, such a decrease may be due to at least three factors. exosmosis of salts; decrease in the size of the cells, and the passage of ions through the bacterial membrane owing to increased permeability.

In our experiments exosmosis seems to be the factor of greatest moment. Our findings that at death there is only a slight decrease in the ability of the bacterial cells suspended in a solution to offer resistance to an electric current makes it appear that any change in permeability of the cell membrane must be of minor value. This is further emphasized by the finding that the dead cells have a lessened ability to obstruct an electric current in that they displace less conducting solution.

In this discussion no values for the resistance of bacterial cells have been assigned, as in such a system the resistances are not in series and the total resistance does not indicate the value of the individual resistances concerned.

SUMMARY

Dead bacterial cells offer resistance to an electric current almost if not equal in amount to that exhibited by live bacterial cells.

Bacterial cells growing in ordinary mediums store up diffusible salts within their bodies to a greater concentration than is found in their habitat.

On the death of bacterial cells by heat or liquor formaldehydi there is an exchange of salts between the cells and the surrounding medium.

In our series of experiments the drop in resistance on cell death could be shown to be due in greatest part to an exosmosis of salts into the surrounding solution.

On death of bacteria there is a definite decrease in the size of the cell.

From our findings it appears that conductivity measurements do not measure a change in permeability of bacterial membranes, but that permeability is only indicated by the exosmosis of salts from the cells killed by heat and by liquor formaldehydi.